

## REMARKS

The Office action mailed 9 May 2011, has been received and its contents carefully noted. The pending claims, claims 29, 30, 35 and 39-43, were rejected and claims 31-34 and 36 were withdrawn from consideration. Reconsideration in view of the following are respectfully requested.

### The Claimed Invention

The claimed invention is directed to a device which is capable of detecting, measuring or monitoring the activities and concentrations of at least one protein having similar or overlapping properties towards multiple substrates in a sample having n number of proteins having similar or overlapping properties towards the plurality of substrates which employs a cartridge comprising a plurality of substrates having n+1 number of substrates. The inclusion of n+1, i.e. one more substrate over the number of proteins, allows the “weighted” calculation of each protein (using the sensitivity coefficients as instantly claimed).

In particular, since the proteins “cross-react” with the plurality of substrates, e.g. both AChE and BChE react with acetylthiocholine and butyrylthiocholine (albeit not to the same degree), any assay using acetylthiocholine to measure AChE will result in a measurement that includes some contribution from BChE, any assay using butyrylthiocholine to measure BChE will result in a measurement that includes some contribution from AChE, and vice versa.

Prior to the present invention, others in the art attempted to overcome the problems of assaying proteins, e.g. AChE and BChE, having cross-reactivity (i.e. similar or overlapping properties) towards multiple substrates by using a substrate that is highly specific for only one of the proteins, e.g. acetyl( $\beta$ -methyl)thiocholine, or by using a selective inhibitor, e.g. ethopropazine, to completely block the activity of one of the proteins, or a combination of both. Other prior art methods also attempted to overcome the problems of cross-reactivity by physically separating a given protein from the other proteins and then assaying the purified given protein. No prior art method or device enables one to calculate the weighted contributions of cross-reactive proteins.

The device according to the present invention enables one to calculate the weighted contributions of cross-reactive proteins. In particular, by using a cartridge that comprises a

plurality of the group having  $n+1$  number of substrates in combination with software which calculates the concentration and/or activity of each protein using a set of equations which encodes for the sensitivity coefficients for each substrate and for each protein and their reaction rates, as set forth in the instant claims, the device according to the present invention, allows one to reliably measure the amount of the activity and/or concentration of a first protein, e.g. BChE, in a sample containing a second protein, e.g. AChE, without guesstimating how much of the amount measured is due to the "overlapping" property of the second protein.

The device according to the present invention also allows one to assay a sample in which it is unknown whether a particular protein is present or not. For example, if using prior art methods and devices to measure protein A in a sample that may or may not have proteins B, C, and D, one would have to (1) separate out protein A, (2) dump in all the inhibitors which are selective for all the possible proteins which might have cross-reactivity with protein A but do not inhibit protein A, or (3) find a specific substrate that only reacts with protein A. Certain circumstances may make the ability to do any one of (1)-(3) challenging or impossible, e.g. often it is challenging or impossible to separate a first protein from second protein when they are highly similar, protein separation techniques often require knowledge about the proteins and the protein of interest being purified away from, and it may be impossible to find inhibitors and/or substrates which are selective for everything but the given protein of interest, especially with cross-reactive proteins.

With the device according to the present invention, one need only use  $n+1$  number of substrates and the sensitivity coefficients to obtain a "weighted" measurement without any worry or care about the proteins in the sample having cross-reactivity towards the substrates being used. In addition, with the sensitivity coefficients in hand (i.e. provided by the software of the device), one need not use any inhibitors when using the claimed device in order to assay one or more proteins having similar or overlapping properties.

#### **Rejection under 35 U.S.C. 103(a)**

The Examiner rejected claims 29, 30, 35 and 39-43 under 35 U.S.C. 103(a) as being unpatentable over London (1995) in view of Worek (1999) and Jacobs (1993). Specifically, the Examiner deemed that Worek discloses the determination of AChE in human blood using two

different substrates, i.e. acetylthiocholine and butyrylthiocholine and that it would have been obvious to modify the device of London to contain at least two substrates in the cartridge.

As previously set forth, nowhere does London teach or suggest using both acetylthiocholine and butyrylthiocholine to measure BChE. Nowhere does London teach or suggest using both acetylthiocholine and butyrylthiocholine to measure AChE. Nowhere does London teach or suggest a device which comprises both acetylthiocholine and butyrylthiocholine. Nowhere does London teach or suggest one device which has a cartridge containing at least two substrates. Nowhere does London teach or suggest accounting for the activity of a second protein in a test sample which overlaps with the activity of the protein of interest by using sensitivity coefficients according to the instant invention. Instead, London teaches using inhibitor As1397 to prevent PCE (i.e. BChE activity) interference when measuring AChE. Thus, based on the teachings of London, one skilled in the art would not have been motivated to (a) provide a cartridge contains acetylthiocholine and butyrylthiocholine in order to measure the activities or concentrations of one cholinesterase, i.e. AChE or BChE, or (b) a cartridge which contains acetylthiocholine, butyrylthiocholine, and one more substrate in order to measure the activities or concentrations of both AChE and BChE in aliquots of the same sample.

Worek does not alleviate the deficiencies of London. Worek explains the problems of prior art protection method, e.g. interference with hemoglobin absorption. Worek references the method of Meuling which uses two substrates to distinguish between AChE and BChE (ref. #23). Worek then explains the methodology for developing the “Worek method”, i.e. measuring AChE by inhibiting BChE with ethopropazine. Worek does use two substrates for measuring both AChE and BChE using a “standard” assay, i.e. Ellman method, for comparison against the “Worek method”. See Table 1 and the corresponding text. Worek then explains in the discussion that the Ellman assay suffers problems of interference from hemoglobin absorbance. Worek also explains the problems of other methods, e.g. separating AChE from BChE and then measuring AChE, and the Meuling method. See p. 87. Worek teaches that the Worek method, which inhibits BChE with ethopropazine (highly specific for BChE), allows the sensitive and precise determination of AChE, i.e. one protein, using only one substrate, i.e. acetylthiocholine.

Nowhere does Worek teach or suggest using n+1 substrates to measure n number of proteins in a given sample. In particular, nowhere does Worek teach or suggest using two

substrates for measuring the activity or concentration of AChE in order to give a “weighted” calculation of AChE in view of the possible presence of BChE. In fact, Worek teaches the use of an inhibitor ethopropazine which completely blocks the activity of BChE in order to obtain a measurement for BChE. Worek does not teach or suggest using two substrates to measure one protein, e.g. AChE. Worek does not teach or suggest using three substrates to measure two proteins, e.g. both AChE and BChE. Thus, Worek does not teach or suggest a method or device which employs the use of n+1 substrates as required by the instant claims.

Worek mentions the Meuling method. Specifically, Worek states “[i]n another approach, 2 substrates were used to distinguish between AChE and BChE [23]”. For the Examiner’s convenience, Applicants submit herewith a copy of Meuling ((1992) Am. J. Indust. Med. A2:231-241).

The Meuling method is Method 2 as set forth on page 243 as follows:

S-butyrylthiocholineiodide was used as a substrate to determine the activity of PsChE in hemolysates ... [and] acetyl(β-methyl)thiocholineiodide was used as substrate to measure AChE activity.

Meuling uses another method, i.e. Method 1, to compare with Method 2. Method 1, as set forth on page 234 is as follows:

[The method] is based on measurement of both cholinesterase activities with the substrate propionylthiocholine. In the hemolysates, the total cholinesterase activity (total-ChE), i.e., the sum of the activities of acetyl and pseudo cholinesterase, is measured first. After inhibiting PsChE with ethopropazine HCl, the same hemolysates are analyzed for AChE activity. Subtracting the latter result from total-ChE yields the activity of PsChE.

As set forth above (and shown in Table I), Method 1 and Method 2 of Meuling use either (1) one substrate and one inhibitor, or (2) two substrates to measure, the activity or concentration of two proteins. Nowhere does Meuling teach or suggest using n+1 substrates to measure n number of proteins. Nowhere does Meuling teach or suggest using two substrates to give a weighted measurement of one protein, e.g. AChE. Nowhere does Meuling teach or suggest using three substrates to obtain weighted measurements of AChE and BChE.

Thus, Worek (and/or Meuling) does not alleviate the deficiencies of London.

As previously set forth, Jacobs merely discloses the Gem Premier system having an analyzer and a dispensable cartridge. Nowhere does Jacobs teach or suggest a method or device

that employs a cartridge having  $n+1$  substrates for assaying  $n$  number of proteins having similar or overlapping properties.

In summary, nowhere do the cited documents, alone or in combination, teach or suggest a method or a device which employs a cartridge having  $n+1$  of substrates for assaying  $n$  number of proteins having similar or overlapping properties towards the substrates. Nowhere do the cited documents, alone or in combination, teach or suggest a method or a device which employs  $n+1$  of substrates and sensitivity coefficients in order to provide weighted measurements for each of the  $n$  number of proteins.

Since the cited documents, alone or in combination, do not teach or suggest a method or device employing  $n+1$  substrates for assaying  $n$  number of proteins, as set forth in the instant claims, the claimed invention is novel and unobvious. Therefore, the rejection under 35 U.S.C. 103(a) should properly be withdrawn.

#### **Request for Rejoinder**

Applicants respectfully request rejoinder of the withdrawn claims which ultimately depend on claim 29.

#### **Request for Interview**

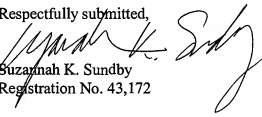
Either a telephonic or an in-person interview is respectfully requested should there be any remaining issues.

### CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Therefore, it is respectfully requested that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, in the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. 1.136(a), and any fees required therefor are hereby authorized to be charged to **Deposit Account No. 210-380**, Attorney Docket No. **034047.003DIV1 (WRAIR 00-23)**.

Respectfully submitted,

  
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